

## Synchronized boll development of *Bt* cotton hybrids and their physiological consequences

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**The temporal and spatial distribution of fruiting forms, their retention and shedding pattern was studied in field-grown *Bt* cotton hybrids MECH 12, MECH 162 and MECH 184 and their non-*Bt* (*NBt*) counterparts at the Central Institute for Cotton Research, Nagpur, India. Plant phenology in terms of time required for squaring and flowering did not vary significantly between *Bt* and *NBt* hybrids. However, boll bursting duration was slightly shorter in *Bt* plants (51 days) compared to *NBt* plants (55 days). *Bt* plants produced nearly 30% less squares, but retained 25% more bolls than *NBt* plants. The initial few sympodial nodes contained most of the open bolls in *Bt* plants, while they were mostly barren in *NBt* plants. This synchronized boll development in *Bt* plants altered the source–sink relationship and led to early crop maturity. Further, this communication discusses why *Bt* plants are more vulnerable to environmental adversities such as drought, waterlogging, cloudy weather, high temperature, etc.**

**Keywords:** *Bt* hybrids, cotton, plant map, square production, synchronized boll load.

REALIZING the importance of severity of the loss of cotton crop due to insect pests, *Bt* cotton (Bollgard) hybrids have been approved for commercial cultivation in India, since March 2002. In most of the experimental trials as well as in farmers' fields, *Bt* hybrids out-yielded non-*Bt* (*NBt*) hybrids<sup>1,2</sup>. *Bt* hybrids are expected to retain more bolls at early growth stage because of better insect control over their *NBt* counterparts. Developing bolls have a great demand for photosynthate and thus plants with higher boll load have a greater inter-organ competition for photo-assimilates<sup>3</sup>. This competition is further aggravated when the availability of photosynthates is limited under conditions such as cloudy weather, water deficit or waterlogging stress, high temperature, etc. and leads to the development of various physiological disorders<sup>4,5</sup>. Thus, both productivity and quality of cotton fibre are affected. Hence, in order to effectively manage the crop it is important to know the temporal and spatial distribution of fruiting forms on the canopy. In this study the temporal and spatial distribution of fruiting forms of *Bt* and *NBt* hybrids were mapped and their influence on source–sink relation and phenology of the crop has been discussed.

*Bt* cotton hybrids MECH 12, MECH 162 and MECH 184 and their *NBt* counterparts were grown at the Central Institute for Cotton Research Farm, Nagpur (21°N and 79°E), Central India under rainfed condition. According to common practice of this region, a spacing of 0.6 m was maintained between the rows and plants, which accommodated a plant population of 27,777 plants/ha. Recommended dose of fertilizer and plant protection measures were applied. Days taken for 50% squaring (out of the total plants observed 50% of the plants had at least one square on them), flowering and maturity were recorded in the plants from middle four rows of each plot. Date of square appearance was recorded on a chart paper along with its position on a sympodia or monopodia. Every alternate day, the monopodia and sympodia were observed and new squares that appeared on the successive positions from the previous date were recorded. Appearance of pinhead squares (approx. 3 mm size) was considered as new squares for counting. The fate of these squares was monitored as long as they were on the plant. The flowering date and boll opening date of each square, which was retained on the plant were noted. The abscised squares on the ground were collected and carefully evaluated to ascertain the causal factor for shedding (physiological or insect attack). Total squares produced, their distribution on the monopodia and sympodia, open flowers and open bolls developed from the squares produced at different stages and shedding pattern of different genotypes were estimated. The time period required for the formation of square to flower and flower to open boll was calculated. Plant mapping was done using temporal and spatial distribution of fruiting forms. Stomatal conductance and photosynthesis of the youngest fully expanded leaf (third from top) was measured with Portable Photosynthesis system (model CIRAS-1, PP systems, UK).

Squaring commenced at around 46 DAS (days after sowing) in *Bt* and *NBt* hybrids. Squares initially appeared on the sympodial nodes and gradually moved upwards in the subsequent positions of the same sympodia as well as in the next sympodia at regular intervals. Squares formed were either retained or shed due to both physiological and entomological factors. Those retained flowered within 22–23 days and matured as open bolls or were shed as green bolls. From flower to boll bursting *Bt* plants required 51 days, while *NBt* plants required a slightly longer period of 55 days (Table 1). From Figure 1 it is clear that the initial few sympodia of *Bt* bore the maximum number of bolls, while boll distribution was random in *NBt* hybrids. *Bt* plants had less number of nodes, plant height and square production. Interestingly, *Bt* plants on an average produced 30% less squares and retained 25% more boll compared to *NBt*. In *Bt* the topmost node to bear the open boll was 24, while the corresponding node in *NBt* was 30. Figure 2 further confirmed that in *Bt*, squares formed on the first and second position of sympodial nodes contributed maximum (45%) to open bolls and farther positions

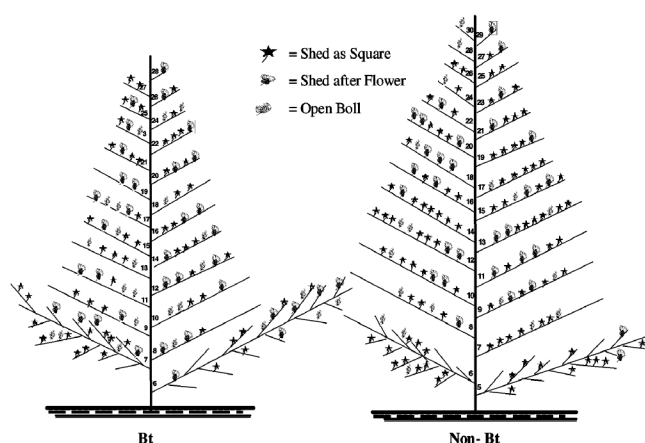
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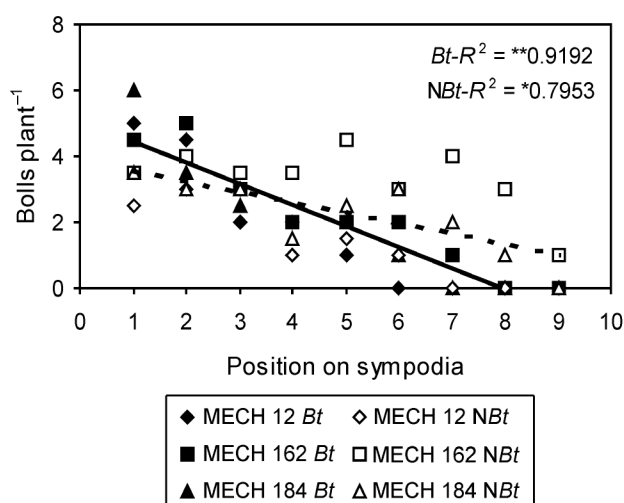
**Table 1.** Plant height, leaf area, dry matter, stomatal conductance and photosynthesis at peak boll development (110 DAS), phenology, square and boll production of *Bt* and *NBt* hybrids

Hybrid	Plant height (cm)	Leaf area (sq. cm plant <sup>-1</sup> )	Dry matter (g plant <sup>-1</sup> )	Stomatal conductance (mol m <sup>-2</sup> s <sup>-1</sup> )	Photosynthesis (μmol m <sup>-2</sup> s <sup>-1</sup> )	Phenology (days)				No. of squares per plant	No. of bolls per plant
						Squaring	Flowering	Boll bursting	Maturity		
MECH-12 <i>Bt</i>	57	5326	108.90	720	34.60	44	22	52	140	52	18
MECH-12 <i>NBt</i>	63	6269	103.05	503	28.30	45	23	56	160	91	14
MECH-162 <i>Bt</i>	70	5118	103.85	740	33.80	48	23	50	170	150	34
MECH-162 <i>NBt</i>	95	5143	97.40	494	29.90	48	26	58	190	184	30
MECH-184 <i>Bt</i>	70	4729	109.65	560	33.10	47	21	50	160	120	28
MECH-184 <i>NBt</i>	73	5821	83.35	483	29.50	47	22	53	180	135	20
Mean <i>Bt</i>	68	5064	106.99	640	33.83	46.5	22	51	160	94	27
Mean <i>NBt</i>	77	5821	93.04	493	29.23	47	23.5	55	180	136	21
CD at 5% genotype	4.89	529	5.24	NS	NS	NS	NS	NS	20	36	4.56
<i>Bt</i> vs <i>NBt</i>	3.46	374	3.71	43	1.36	NS	NS	3.0	30	25	3.0
Gen × <i>Bt</i>	6.93	631	7.41	65	3.52	NS	NS	NS	40	41	6.0

NS, Non-significant.



**Figure 1.** Temporal and spatial distribution of squares, flowers and open bolls of a typical *Bt* and *NBt* plant grown under field condition.



**Figure 2.** Open bolls at different positions of sympodial nodes of *Bt* (thick line) and *NBt* (dashed line) cotton hybrids.

showed a sharp decline in boll number. On the other hand, in *NBt* almost uniform boll distribution was found up to the eighth position on the sympodia. Thus, the basal sympodia of *Bt* had a good number of bolls, while in *NBt* they were mostly barren. The better control of insects in *Bt* saved these basal fruiting forms and resulted in more synchronized boll development compared to *NBt* plants.

The developing bolls have a great demand for photosynthate and other nutrients. This demand is met by both current photosynthate as well as translocation of reserved carbohydrate from other parts, especially older leaves<sup>5,6</sup>. At 110 DAS, significantly higher stomatal conductance and photosynthesis was observed in *Bt* plants, which were mainly driven by the high boll load. This led to significantly higher biomass accumulation in *Bt* plants over their *NBt* counterparts, as reported earlier<sup>7</sup>. However, this period also coincides with the end of rainy season (September end) and beyond this period plant growth is limited by moisture and nutrients. Thus, in *Bt* higher boll load coupled with the limitation of moisture and nutrients led to stiff interorganal competition for photosynthates which resulted in senescence, early cessation of growth, reduced the new flushes of leaves and squares and increased the shedding of young fruiting forms. This was evident from the fact that at this stage the leaf area of *Bt* plants was significantly lower than *NBt* plants (Table 1). Earlier workers also reported similar findings under rainfed condition<sup>8</sup>. The last boll to mature in *Bt* plant was from squares which were formed in the third week of September, while in *NBt* it was from squares formed in mid-October. Thus, *Bt* plants had an early maturity (cut-out) at least by 20 days compared to *NBt* plants.

Under rainfed condition the crop is often subjected to environmental adversities such as cloudy weather, water deficit or waterlogging stress, high temperature, etc. These limit the supply of photosynthates to the developing organs. Since boll development is more synchronized in *Bt*,

any of these adversities would develop some of the physiological disorders such as leaf reddening or senescence, square and boll shedding, parawilt or suddenwilt, bad opening of bolls, etc. Quite often these disorders are reported in farmers' fields. This understanding of temporal and spatial distribution of fruiting forms in *Bt* and *NBt* hybrids might help in evolving efficient physical, chemical or biological management strategies to overcome the above disorders.

1. Mohan, K. S. and Manjunath, T. M., *Bt* cotton – India's first transgenic crop. *J. Plant Physiol.*, 2002, **29**, 225–236.
2. Ramasundaram, P., The performance of *Bt* cotton hybrids in India. *ICAC Rec.*, 2005, **23**, 17–18.
3. Guinn, G., Fruiting of cotton. III. Nutritional stress and cutout. *Crop Sci.*, 1985, **25**, 981–985.
4. Stewart, S. D. and Sterling, W. L., Susceptibility of cotton fruiting forms to insects, boll rot and physical stress. *J. Econ. Entomol.*, 1989, **82**, 593–598.
5. Constable, G. A., Mapping the production and survival of fruit on field grown cotton. *Agron. J.*, 1991, **83**, 374–378.
6. Guinn, G., Abscission of cotton floral buds and bolls as influenced by factors affecting photosynthesis and respiration. *Crop Sci.*, 1974, **14**, 291–293.
7. Hebbar, K. B., Perumal, N. K. and Khadi, B. M., Photosynthesis and plant growth response of transgenic *Bt* cotton (*Gossypium hirsutum* L.) hybrids under field condition. *Photosynthetica*, 2007, **45**, 254–258.
8. Bhatt, J. G., In *Cotton Physiology* (eds Sundaram, D. V. and Rao, S. B. P.), Cotton Monograph Series, Indian Society for Cotton Improvement, Mumbai, 1996, pp. 26–37.

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## Self-rotation of an assembly of two or more cylindrical objects in optical tweezers: A simple approach for realization of optically driven micromotors

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**We show that an assembly of two or more cylindrical objects rotates by itself when trapped in conventional optical tweezers. The rotational speed and direction of rotation were observed to depend on the asymmetry of the structure, and for a given structure the speed was found to increase linearly with increase in the trapping**

**beam power. A ray optic model was also developed for the assembly of rods. Its predictions on the direction of rotation and the optical torque on the assembly were in qualitative agreement with the experimental observations. Such an assembly of rods may provide a convenient and dynamically reconfigurable approach for optically driven micromotors.**

**Keywords:** Microfluidics, micromotors, optical trapping, optical tweezers.

THERE exists considerable interest on the development of optically driven micromotors as these could be driven remotely by laser beams and thus could play an important role in various microfluidic applications. A variety of methods have therefore been developed for rotation of optically trapped objects<sup>1–5</sup>. One approach actively pursued towards this objective is to fabricate structures that experience windmill-type torque by transfer of linear momentum from the trapping beam and thus rotate<sup>6,7</sup>. However, fabrication of such special structures is complicated and costly, and may limit the use of this approach. We show in this communication that an assembly of two or more cylindrical objects provides a convenient and dynamically reconfigurable approach for optically driven micromotors. Further, the individual rods can be transported through narrow channels (with width smaller than the size of the micromotor), either by microfluidic flow or by laser tweezers to the desired location where they can be assembled to construct micromotors and dismantled after use. We also describe a ray optic model for the assembly, which accurately predicts the direction of rotation as well as dependence of the torque on the asymmetry of the structure.

We used a conventional inverted optical tweezers set-up for studies on the dynamical behaviour of an assembly of cylindrical rods when subjected to a trapping beam. The output of a 1064 nm, TEM<sub>00</sub> mode, cw Nd : YAG laser (Solid State Laser Division, Raja Ramanna Centre for Advanced Technology, Indore, India) was coupled to a 100X/1.3 microscope objective (Carl Zeiss GmbH, Jena, Germany) and used as the trapping beam. The sequence of digitized images, acquired using a CCD camera and frame-grabber, was used for studying the dynamical behaviour of the assemblies of micro-rods. To prevent the backscattered laser light from reaching the CCD detector, an IR cut-off filter was used. The laser beam power was measured at the back aperture of the microscope objective using a power meter (Coherent Inc., USA). The trapping beam power at the sample plane was estimated using the transmission factor of the objective (57%) that was determined using the dual objective technique described by Misawa *et al.*<sup>8</sup>. The glass rods (Nippon Electric Glass Co Ltd, Japan) used in the study had diameter of ~5 μm and lengths varying from 10 to 30 μm.

It is known<sup>9–11</sup> that when placed in a point optical tweezers, a cylindrical rod gets aligned along the axis of

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